Naval Research Laboratory
Washington, DC 20375-5320

NRL/6920/MR—2023/1

Persistence of Venezuelan Equine Encephalitis Virus on Exposure to Three Types of Materials

ANNE KUSTERBECK (FORMER NRL EMPLOYEE)

Center for Bio/Molecular Science and Engineering

JINNY LIU

ELLEN GOLDMAN

Laboratory for Biomaterials and Systems
Center for Bio/Molecular Science and Engineering

EDWARD GORZKOWSKI

HEONJUNE RYOU

SYED B. QADRI

JAMES WOLLMERSHAUSER

Multifunctional Materials Branch
Materials Science and Technology Division

February 15, 2023

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
15-02-2023	NRL Memorandum Report	07/15/2020 – 12/31/2020

4. TITLE AND SUBTITLE

Persistence of Venezuelan Equine Encephalitis Virus on Exposure to Three Types of Materials

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
0602236N

6. AUTHOR(S)

Anne Kusterbeck*, Ellen Goldman, Jinny Liu, Edward Gorzkowski, Heonjune Ryou, Syed Qadri, and James Wollmershauser

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
1U31

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Research Laboratory
4555 Overlook Avenue, SW
Washington, DC 20375-5320

8. PERFORMING ORGANIZATION REPORT NUMBER

NRL/6920/MR--2023/1

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
One Liberty Center
875 N Randolph Street
Arlington, VA 22217-1958

10. SPONSOR / MONITOR'S ACRONYM(S)
ONR

11. SPONSOR / MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
*Former NRL Employee Retired

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Ellen Goldman
U	U	U	U	12	**19b. TELEPHONE NUMBER** *(include area code)* (202) 404-6052

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

This page intentionally left blank.

Introduction

As many were made acutely aware during the initial period of the COVID 19 pandemic in the spring of 2020, there is the potential for viral infection through fomites, inanimate objects can be contaminated with pathogens. Although transmission through fomites was shown to be only a minor component of the SARS-CoV-2 pandemic [1], this mode of transmission can be more prevalent with other viruses [2, 3]. Understanding the persistence of viruses in the environment by assessing virus viability in different types of samples, such as liquid, aerosol, and surfaces, can be important in formulating infection control both for naturally occurring outbreaks as well as in the case of an intentional release of a biothreat agent [4].

Alphaviruses are enveloped viruses with RNA genomes and include viruses of importance to human and animal health as well as ones that have been identified as potential biothreat agents [5]. There are only limited studies on the persistence of alphaviruses. In one study Chikungunia virus was shown to be infective for over 60 weeks when stored refrigerated in sterile distilled water in the dark [6]. Likewise Venezuelan equine encephalitis virus (VEEV) was stable in distilled water at 4 °C with essentially no decrease over 21 days, while when samples were kept at room temperature (21 °C) there was a steady decrease in viability over the three weeks [7]. Another study examined the persistence of VEEV and other viruses of concern when dried onto solid surfaces. This work showed that the VEEV was more resistant to deactivation than either Lassa virus or Ebola virus, with 90% reduction in viral load on a glass surface at 98 hours; the loss of VEEV viability was similar on glass, metal, and rubber surfaces [8]

We examined the persistence of VEEV after exposure to three different types of materials: natural pumice, activated carbon, and copper foam. These materials were chosen as they have the potential to be compatible with air filtration. For each of the materials we examined at least two different grades/porosities, and tracked the infectivity of VEEV exposed to the materials. This work adds to the data on the persistence of VEEV, and may provide information about the ability of these surfaces to inactivate viruses.

Material Characterization

Three materials types were evaluated for their efficacy at inactivating viruses, including natural pumice, activated carbon, and copper foam in two conditions (exposed to water vapor and with the surface oxide reduced). For each material type, the specific surface area (i.e. porosity) was also varied to explore the influence on the amount of material surface available to virus on the virus survival (for a constant viral loading).

Manuscript approved January 17, 2023.

Two grades of natural pumice were studied; Hess Grade 3/4 x 3/8 and Hess Grade 3/8 x #8. While Hess Grade 3/8 x #8 has a slightly smaller manufacturer quote primary particle size, both are expected to have high porosity levels which consists of large-scale porosity and a natural ultra-fine nano-porosity. Figure 1 shows Scanning Electron Microscopy (SEM) of the pumice surfaces showing that porosity exists at various length scales, ranging from the 10's of microns down to the nanometer scale. Imaging on these samples was performed on a fractured surface of the pumice in low vacuum mode (30 Pa). The low vacuum mode allowed SEM imaging without a conductive coating on the samples. There was no notable difference in the SEM observable microstructure between the two pumice grades.

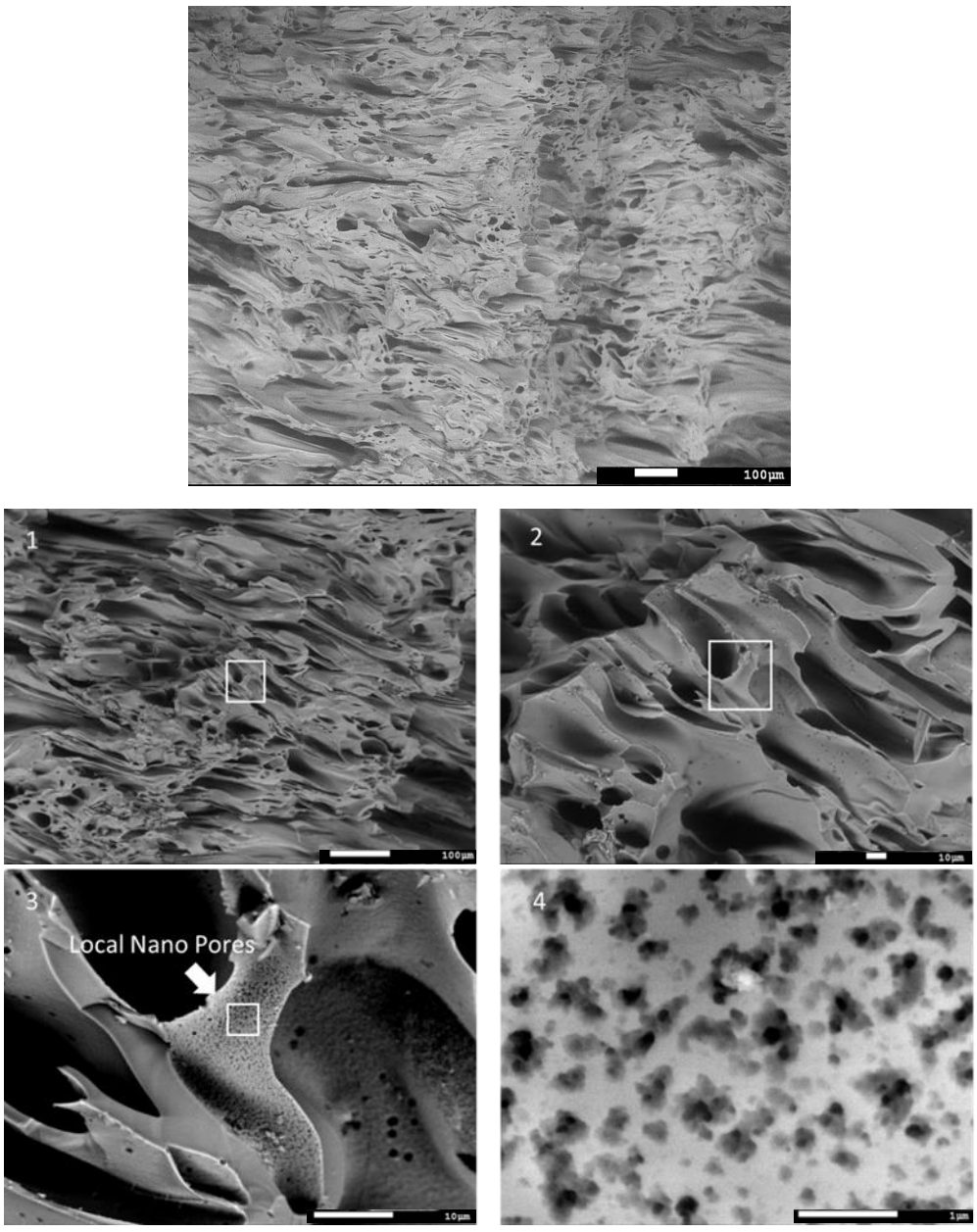

Figure 1: Scanning Electron Microscopy (SEM) of Hess natural pumice surface (Hess Grade 3/4 x 3/8).

The size of the nanoporosity was quantitatively determined from X-ray diffraction analysis. Using X-ray diffractometry (XRD) performed in the region of so-called first sharp diffraction peak (FSDP), the nanovoid structure can be determined in light of the Elliott model [9, 10]. This FSDP can be distinctly observed in 2 θ/ θ scans using Cu Kα radiation.

Within Elliott's model, the interlayer separation, quasi-periodic in nature with an effective periodicity, R, can be calculated from the position of the FSDP in respect to the magnitude of the scattering vector Q1:

$Q1=4\pi\sin\theta/\lambda$ and $R\approx 2\pi/Q1$

The correlation length, L, over which quasi-periodic, real-space density fluctuations take place, can be calculated from the full width at half maximum (FWHM) ΔQ1 of the FSDP: $L=2\pi/\Delta Q1$.

Figure 2 shows the FSDP XRD pattern for the two pumice grades. Based on the calculations using the above equations we have calculated the diameters of the fine scale voids (porosity) to be 7 Å and 6 Å for Hess Pumice 3/8 x #8 and 3/4 x 3/8, respectively.

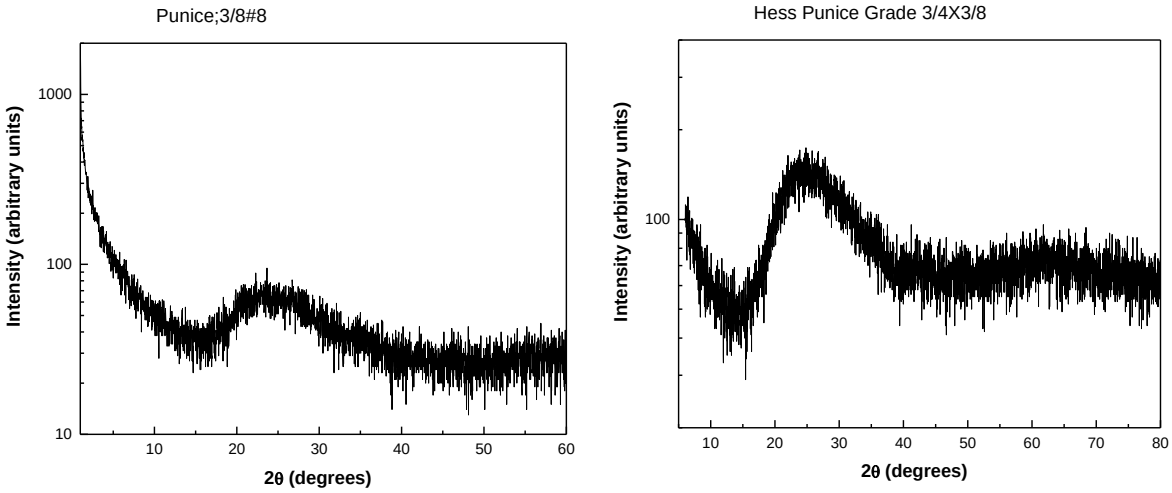

Figure 2: FSDP XRD pattern for the two pumice grades

For activated carbon materials, activated carbon felts with 4 different surface area levels were explored; CeraMaterials Activated Carbon Felt ACF1000, ACF1300, ACF1500, and ACF1600. ACF1000 has the lowest surface area and ACF 1600 has the highest surface area. SEM micrographs of ACF1300 and ACF1600 are shown in Figure 3. The felts are shown to be fiber matts with different fiber thicknesses where the higher surface area felts have smaller fiber diameters. Nanoporosity within the fibers is not observed in SEM micrographs, nor is it expected to exist in the felt fiber matts.

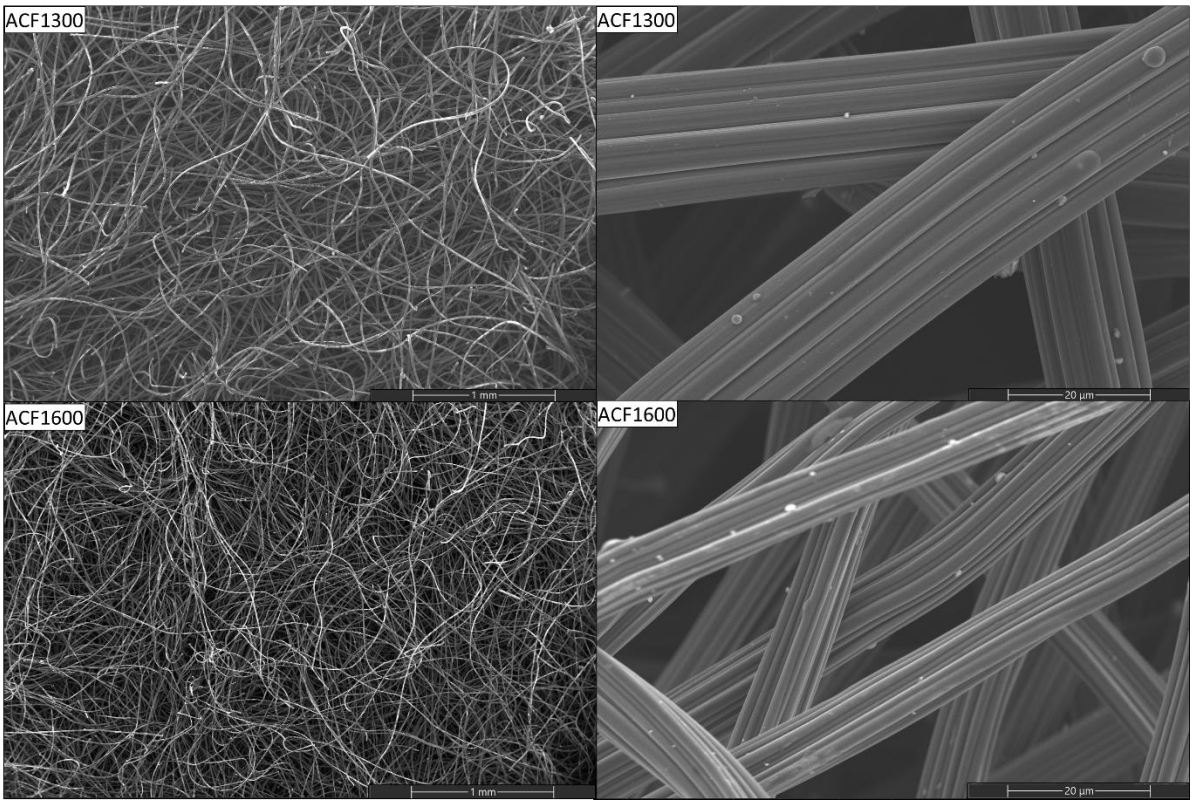

Figure 3: SEM micrographs of ACF1300 and ACF1600 CeraMaterials Carbon Felt

For copper, copper foams with 2 different porosity levels were explored; ERG's Duocel Copper foam (40 pores per inch) compressed by 27.7% and 65.7% to vary to exposed surface area (i.e., effective porosity). It is expected that the copper foams will form a copper oxide film on the surface of the foam structure during the autoclave step prior to viral studies. Therefore, a set of copper foams were treated in forming gas (10% H2/90% Argon) at 450°C as a sterilization step in lieu of autoclave sterilization and stored in argon without exposure to ambient. SEM micrographs of two of the copper foams are shown in Figure 4; ERG Duocel Copper foam compressed by 27.7% and 65.7%. The foam compressed to 27.7% has significant open porosity, while the open channels in foam compressed to 65.7% are much smaller-potentially provided less surface area for viral interaction. Similar to the carbon felt, Nanoporosity within the foam matrix is not observed in SEM micrographs, nor is it expected to exist.

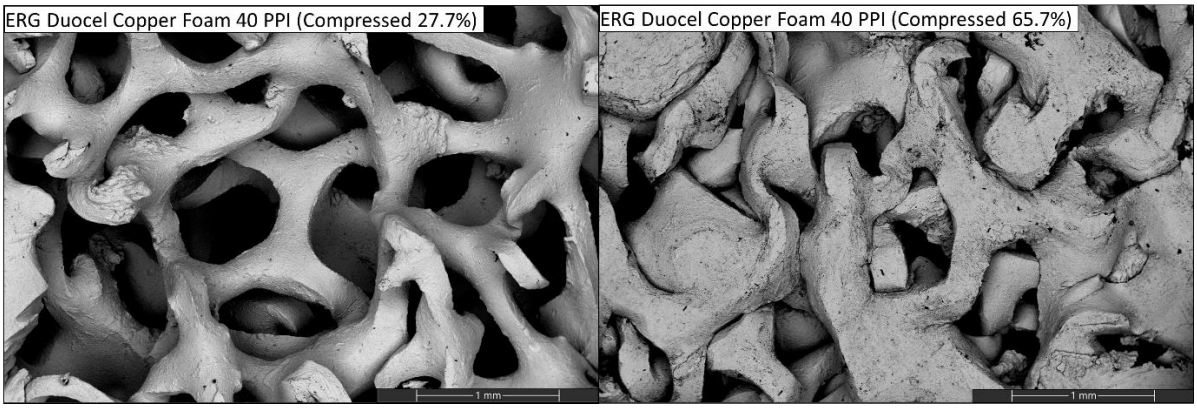

Figure 4: SEM micrographs of two copper foams.

Viral persistence on exposure to different materials

First each of the materials was subject to sterilization by autoclave or as described above for the copper foam. We used the vaccine strain of VEEV (TC83) for these experiments.

To assess viral infectivity, approximately 1500 plaque forming units/mL of TC83 in culture medium supplemented with 2% fetal bovine serum and antibiotics were incubated with the material for various time points up to 48 hours at room temperature, 22 °C with gently shaking. The virus solution at each time point was plated on Vero cell monolayers and stained with neutral red for 18 hours as described previously [11]. The titer was measured by counting plaques in each well. The percent of viral survival is the plaque numbers at each time point divided by the number of plaques at 0 hours. Control samples contains only virus without added material. At least two biological replicates of each condition were performed and the average and standard deviation are plotted.

We evaluated the effect of the two grades of pumice, 4 carbon felts and 4 Cu foams on VEEV TC83 viability up to 48 hours. Results from the pumice are shown in Figure 5. Our data indicate that the titer of TC 83 decreased to less than 50% in the first 6 hours and less than 20% in 24 hours for both grades of pumice. We examined three different sizes of pumice particles by weight, ranging from 0.11 to 0.24 g, for each grade. All three pumice particles for each grade consistently show the decreased survival rate relative to the control. In all cases, the survival titer was much lower in grade 3/8x#8, suggesting the larger surface area for 3/8x#8 (larger size for this grade) inactivates more viruses as reflected in the lower titer.

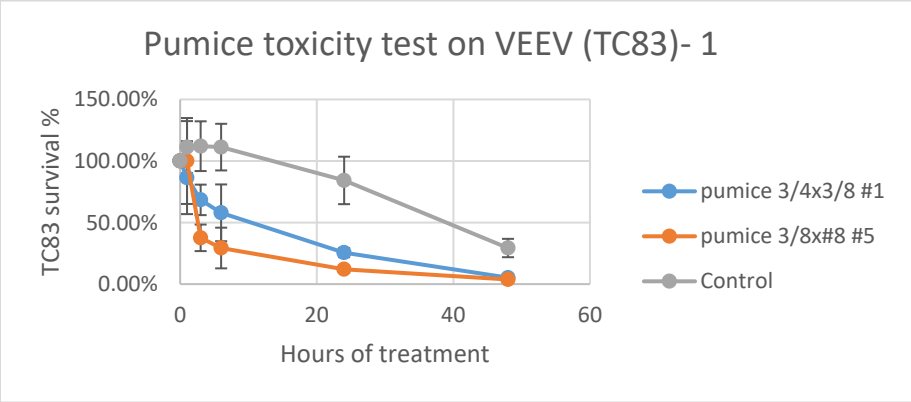

Figure 5: Measurement of pumice toxicity effect on TC83 titers. Approximately 200 TC83 viral particles were mixed with pumice samples for 0, 1, 3, 6, 24, and 48 hours. Following incubation, the number of plaque forming units (pfu) was measured on host cells, Vero cells. The percentage was normalized to the pfu numbers obtained from 0 hours.

Activated Carbon Felt, ACF1000, ACF1300, ACF1500, and ACF1600 were mixed with TC83 up to 48 hours for the initial test. Each of these materials has different surface areas, with ACF1000 having the lowest and ACF1600 the highest. The carbon felts absorb the viral solution, so we shortened the mixing time to 6 hours as indicated in Figure 6. Our data suggests that only the carbon felt with the highest surface area ,ACF1600, significantly reduced the viability of the TC83 compared to the control.

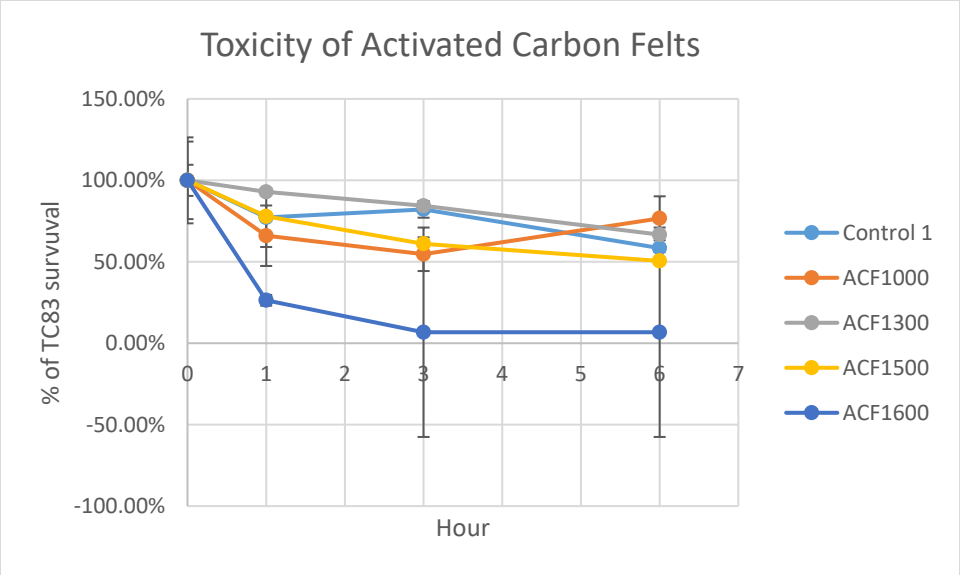

Figure 6: Assessment of toxicity of activated carbon felts on TC83 titer. Four types of carbon felts treated with different temperatures and the same size of a small sheet from each type was mixed with the same amount of TC83 and the titer was measured at 0, 1, 3, 6 hours.

Copper foams with two porosities (40 pores per inch compressed to 27.7% and 65.7% dense) were examined. First the copper foam samples were sterilized using two different methods; one set treated in forming gas (10% H2/90% Argon) at 450°C and the other autoclaved. Materials from each procedure were used to mix with VEEV TC83 for up to 48 hours in the initial tests. In these tests, we observed no TC83 survival at the first time point (1hour). We redid the experiment over a shorter time course of up to 90 minutes as indicated in Figure 7, looking at virus viability at 0, 5, 15, 30, 60 and 90 minutes. Our data show that both copper foams with low and high porosity contents completely inactivated TC83 in 30 minutes. The 27.7% sample sterilized by the gas treatment appears to have slightly increased the toxicity of copper to TC83, however this will need to be examined in a larger number of samples to determine if this difference is reproducible.

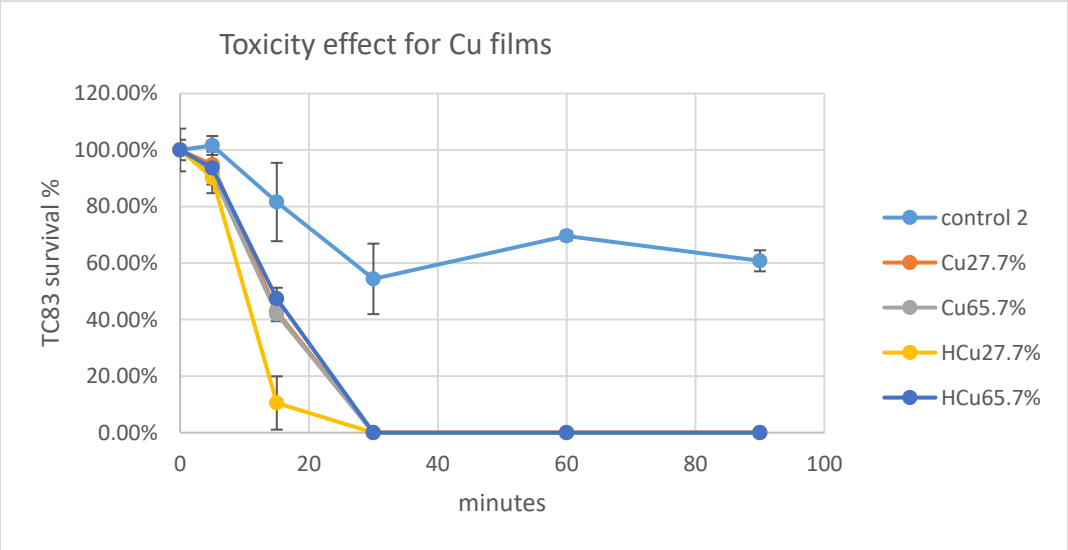

Figure 7: Toxicity measurement for copper foam. Copper foam treated with forming gas at 450 °C is abbreviated as HCu. For each sterilization method, copper foam with two different porosities were mixed with VEEV TC83 and the titer was measured at 0, 5, 15, 30, 60, and 90 minutes.

Discussion and Conclusions

Overall, we have seen the toxicity effects of different materials on VEEV strain TC83 viability. With both the pumice samples and carbon felt, the material with the larger surface area was more effective at inactivating the virus suggesting that use a "filtration" material will increase effectiveness as a viral suppressant. Our findings are consistent with studies that have shown decreased persistence of enveloped viruses on porous surfaces, such as curtains or cotton gowns, versus stainless steel or plastic [12, 13]. Among the types of samples tested, copper most significantly inactivated viruses. Our result suggesting that the alphavirus can only survive on copper surface less than 1 hr, is consistent with other scientific reports that Cu is quite toxic to microorganisms [14, 15].

We show preliminary evidence that exposure to natural pumice may have utility in reducing viral persistence. It is not clear if the pumice material were truly toxic to the VEEV TC83 or if the virus is adsorbing to the surface as adsorption could also explain the reduced titer. Similarly the virus might have adsorbed to the activated carbon felt sample that showed a decrease in viable virus. Nonetheless, capturing virus by adsorption has the potential to inhibit spread of viruses. Many factors influence viral viability, such as temperature, exposure to sunlight, and absolute humidity. A systematic study of the pumice and carbon felt, including recovery of viruses dried on material at controlled temperature and humidity will be needed to determine their utility for viral inactivation.

Acknowledgements

This research was sponsored by the Office of Naval Research.

References:

1. Goldman E, Elkins Christopher A. SARS Wars: the Fomites Strike Back. Appl Environ Microbiol. 87(13):e00653-21. doi: 10.1128/AEM.00653-21.

2. Stephens B, Azimi P, Thoemmes MS, Heidarinejad M, Allen JG, Gilbert JA. Microbial Exchange via Fomites and Implications for Human Health. Current Pollution Reports. 2019;5(4):198-213. doi: 10.1007/s40726-019-00123-6.
3. Kraay ANM, Hayashi MAL, Hernandez-Ceron N, Spicknall IH, Eisenberg MC, Meza R, et al. Fomite-mediated transmission as a sufficient pathway: a comparative analysis across three viral pathogens. Bmc Infect Dis. 2018;18(1):540. doi: 10.1186/s12879-018-3425-x.
4. Sinclair R, Boone Stephanie A, Greenberg D, Keim P, Gerba Charles P. Persistence of Category A Select Agents in the Environment. Appl Environ Microbiol. 2008;74(3):555-63. doi: 10.1128/AEM.02167-07.
5. Nagata LP, Wong JP, Hu W-g, Wu JQ. Vaccines and therapeutics for the encephalitic alphaviruses. Future Virology. 2013;8(7):661-74. doi: 10.2217/fvl.13.42.
6. Pezzi L, Py O, Gilles M, Saba Villarroel PM, Diarra I, Fourié T, et al. Long-Term Infectivity of Chikungunya Virus Stored in the Dark at 4°C. Vector-Borne and Zoonotic Diseases. 2021;21(12):989-93. doi: 10.1089/vbz.2021.0061.
7. Fitzgibbon JE, Sagripanti JL. Analysis of the survival of Venezuelan equine encephalomyelitis virus and possible viral simulants in liquid suspensions. Journal of Applied Microbiology. 2008;105(5):1477-83. doi: https://doi.org/10.1111/j.1365-2672.2008.03919.x.
8. Sagripanti J-L, Rom AM, Holland LE. Persistence in darkness of virulent alphaviruses, Ebola virus, and Lassa virus deposited on solid surfaces. Arch Virol. 2010;155(12):2035-9. doi: 10.1007/s00705-010-0791-0.
9. Elliott SR. Medium-range structural order in covalent amorphous solids. Nature. 1991;354(6353):445-52. doi: 10.1038/354445a0.
10. Elliott SR. Extended-range order, interstitial voids and the first sharp diffraction peak of network glasses. Journal of Non-Crystalline Solids. 1995;182(1):40-8. doi: https://doi.org/10.1016/0022-3093(94)00539-7.
11. Liu JL, Zabetakis D, Gardner CL, Burke CW, Glass PJ, Webb EM, et al. Bivalent single domain antibody constructs for effective neutralization of Venezuelan equine encephalitis. Scientific Reports. 2022;12(1):700. doi: 10.1038/s41598-021-04434-x.
12. Whitworth C, Mu Y, Houston H, Martinez-Smith M, Noble-Wang J, Coulliette-Salmond A, et al. Persistence of Bacteriophage Phi 6 on Porous and Nonporous Surfaces and the Potential for Its Use as an Ebola Virus or Coronavirus Surrogate. Appl Environ Microbiol. 86(17):e01482-20. doi: 10.1128/AEM.01482-20.
13. Lai MYY, Cheng PKC, Lim WWL. Survival of Severe Acute Respiratory Syndrome Coronavirus. Clinical Infectious Diseases. 2005;41(7):e67-e71. doi: 10.1086/433186.
14. Vincent M, Duval RE, Hartemann P, Engels-Deutsch M. Contact killing and antimicrobial properties of copper. Journal of Applied Microbiology. 2018;124(5):1032-46. doi: https://doi.org/10.1111/jam.13681.
15. Govind V, Bharadwaj S, Sai Ganesh MR, Vishnu J, Shankar KV, Shankar B, et al. Antiviral properties of copper and its alloys to inactivate covid-19 virus: a review. BioMetals. 2021;34(6):1217-35. doi: 10.1007/s10534-021-00339-4.